

WHAT IS CLAIMED IS:

- 1 1. A receptor recognition factor implicated in the transcriptional stimulation of
2 genes in target cells in response to the binding of a specific polypeptide ligand to
3 its cellular receptor on said target cell, said receptor recognition factor having the
4 following characteristics:
 - 5 a) apparent direct interaction with the ligand-bound receptor and
6 activation of one or more transcription factors capable of binding with a specific
7 gene;
 - 8 b) an activity demonstrably unaffected by the presence or concentration
9 of second messengers;
 - 10 c) direct interaction with tyrosine kinase domains; and
 - 11 d) a perceived absence of interaction with G-proteins.
- 1 2. The receptor recognition factor of Claim 1 which is proteinaceous in
2 composition.
- 1 3. The receptor recognition factor of Claim 1 which is cytoplasmic in origin.
- 1 4. The receptor recognition factor of Claim 1 which is a polypeptide having
2 an amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ
3 ID NO:10 and SEQ ID NO:12.
- 1 5. The receptor recognition factor of Claim 1 which is derived from
2 mammalian cells.
- 1 6. The receptor recognition factor of Claim 1 labeled with a detectable label.
- 1 7. The receptor recognition factor of Claim 6 wherein the label is selected
2 from enzymes, chemicals which fluoresce and radioactive elements.

1 8. An antibody to a receptor recognition factor, the factor to which said
2 antibody is raised having the following characteristics:

3 a) apparent direct interaction with the ligand-bound receptor and
4 activation of one or more transcription factors capable of binding with a specific
5 gene;

6 b) an activity demonstrably unaffected by the presence or concentration
7 of second messengers; and

8 c) direct interaction with tyrosine kinase domains; and

9 d) a perceived absence of interaction with G-proteins.

1 9. The antibody of Claim 8 which is a polyclonal antibody.

1 10. The antibody of Claim 8 which is a monoclonal antibody.

1 11. An immortal cell line that produces a monoclonal antibody according to
2 Claim 10.

1 12. The antibody of Claim 8 labeled with a detectable label.

1 13. The antibody of Claim 12 wherein the label is selected from enzymes,
2 chemicals which fluoresce and radioactive elements.

1 14. A DNA sequence or degenerate variant thereof, which encodes a receptor
2 recognition factor, or a fragment thereof, selected from the group consisting of:

3 (A) the DNA sequence of FIGURE 1;

4 (B) the DNA sequence of FIGURE 14;

5 (C) the DNA sequence of FIGURE 15;

6 (D) DNA sequences that hybridize to any of the foregoing DNA
7 sequences under standard hybridization conditions; and

8 (E) DNA sequences that code on expression for an amino acid sequence
9 encoded by any of the foregoing DNA sequences.

- 1 15. A recombinant DNA molecule comprising a DNA sequence or degenerate
2 variant thereof, which encodes a receptor recognition factor, or a fragment
3 thereof, selected from the group consisting of:
4 (A) the DNA sequence of FIGURE 1;
5 (B) the DNA sequence of FIGURE 14;
6 (C) the DNA sequence of FIGURE 15;
7 (D) DNA sequences that hybridize to any of the foregoing DNA
8 sequences under standard hybridization conditions; and
9 (E) DNA sequences that code on expression for an amino acid sequence
10 encoded by any of the foregoing DNA sequences.
- 1 16. The recombinant DNA molecule of either of Claims 14 or 15, wherein said
2 DNA sequence is operatively linked to an expression control sequence.
- 1 17. The recombinant DNA molecule of Claim 16, wherein said expression
2 control sequence is selected from the group consisting of the early or late
3 promoters of SV40 or adenovirus, the lac system, the trp system, the TAC system,
4 the TRC system, the major operator and promoter regions of phage λ , the control
5 regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the
6 promoters of acid phosphatase and the promoters of the yeast α -mating factors.
- 1 18. A probe capable of screening for the receptor recognition factor in alternate
2 species prepared from the DNA sequence of Claim 14.
- 1 19. A unicellular host transformed with a recombinant DNA molecule
2 comprising a DNA sequence or degenerate variant thereof, which encodes a
3 receptor recognition factor, or a fragment thereof, selected from the group
4 consisting of:
5 (A) the DNA sequence of FIGURE 1;
6 (B) the DNA sequence of FIGURE 14;

- 7 (C) the DNA sequence of FIGURE 15;
8 (D) DNA sequences that hybridize to any of the foregoing DNA
9 sequences under standard hybridization conditions; and
10 (E) DNA sequences that code on expression for an amino acid sequence
11 encoded by any of the foregoing DNA sequences;
12 wherein said DNA sequence is operatively linked to an expression control
13 sequence.

1 20. The unicellular host of Claim 19 wherein the unicellular host is selected
2 from the group consisting of E. coli, Pseudomonas, Bacillus, Streptomyces,
3 yeasts, CHO, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, and BMT10 cells,
4 plant cells, insect cells, and human cells in tissue culture.

1 21. A method for detecting the presence or activity of a receptor recognition
2 factor, said receptor recognition factor having the following characteristics:
3 apparent direct interaction with the ligand-bound receptor and activation of one or
4 more transcription factors capable of binding with a specific gene; an activity
5 demonstrably unaffected by the presence or concentration of second messengers;
6 direct interaction with tyrosine kinase domains; and a perceived absence of
7 interaction with G-proteins, wherein said receptor recognition factor is measured
8 by:

9 A. contacting a biological sample from a mammal in which the
10 presence or activity of said receptor recognition factor is suspected with a binding
11 partner of said receptor recognition factor under conditions that allow binding of
12 said receptor recognition factor to said binding partner to occur; and

13 B. detecting whether binding has occurred between said receptor
14 recognition factor from said sample and the binding partner;

15 wherein the detection of binding indicates that presence or activity of said
16 receptor recognition factor in said sample.

1 22. A method for detecting the presence and activity of a polypeptide ligand
2 associated with a given invasive stimulus in mammals comprising detecting the
3 presence or activity of a receptor recognition factor according to the method of
4 Claim 21, wherein detection of the presence or activity of the receptor recognition
5 factor indicates the presence and activity of a polypeptide ligand associated with a
6 given invasive stimulus in mammals.

1 23. The method of Claim 22 wherein said invasive stimulus is an infection.

1 24. The method of Claim 22 wherein said invasive stimulus is selected from
2 the group consisting of viral infection, protozoan infection, tumorous mammalian
3 cells, and toxins.

1 25. A method for detecting the binding sites for a receptor recognition factor,
2 said receptor recognition factor having the following characteristics:

3 apparent direct interaction with the ligand-bound receptor and activation of
4 one or more transcription factors capable of binding with a specific gene;

5 an activity demonstrably unaffected by the presence or concentration of
6 second messengers;

7 direct interaction with tyrosine kinase domains; and

8 a perceived absence of interaction with G-proteins; wherein the binding
9 sites for said receptor recognition factor are measured by:

10 A. placing a labeled receptor recognition factor sample in
11 contact with a biological sample from a mammal in which binding sites for said
12 receptor recognition factor are suspected;

13 B. examining said biological sample in binding studies for the
14 presence of said labeled receptor recognition factor;

15 wherein the presence of said labeled recognition factor indicates a binding
16 site for a receptor recognition factor.

1 26. A method of testing the ability of a drug or other entity to modulate the
2 activity of a receptor recognition factor which comprises

3 A. culturing a colony of test cells which has a receptor for the
4 receptor recognition factor in a growth medium containing the receptor recognition
5 factor;

6 B. adding the drug under test; and

7 C. measuring the reactivity of said receptor recognition factor with the
8 receptor on said colony of test cells,

9 wherein said receptor recognition factor has the following characteristics:

10 a) apparent direct interaction with the ligand-bound receptor and
11 activation of one or more transcription factors capable of binding with a specific
12 gene;

13 b) an activity demonstrably unaffected by the presence or concentration
14 of second messengers;

15 c) direct interaction with tyrosine kinase domains; and

16 d) a perceived absence of interaction with G-proteins.

1 27. An assay system for screening drugs and other agents for ability to
2 modulate the production of a receptor recognition factor, comprising:

3 A. culturing an observable cellular test colony inoculated with a drug
4 or agent;

5 B. harvesting a supernatant from said cellular test colony; and

6 C. examining said supernatant for the presence of said receptor
7 recognition factor wherein an increase or a decrease in a level of said receptor
8 recognition factor indicates the ability of a drug to modulate the activity of said
9 receptor recognition factor, said receptor recognition factor having the following
10 characteristics:

11 a) apparent direct interaction with the ligand-bound receptor and
12 activation of one or more transcription factors capable of binding with a specific
13 gene;

- 14 b) an activity demonstrably unaffected by the presence or concentration
- 15 of second messengers;
- 16 c) direct interaction with tyrosine kinase domains; and
- 17 d) a perceived absence of interaction with G-proteins.

- 1 28. A test kit for the demonstration of a receptor recognition factor in a
- 2 eukaryotic cellular sample, comprising:
- 3 A. a predetermined amount of a detectably labelled specific binding
- 4 partner of a receptor recognition factor, said receptor recognition factor having the
- 5 following characteristics: apparent direct interaction with the ligand-bound receptor
- 6 and activation of one or more transcription factors capable of binding with a
- 7 specific gene; an activity demonstrably unaffected by the presence or concentration
- 8 of second messengers; direct interaction with tyrosine kinase domains; and a
- 9 perceived absence of interaction with G-proteins;
- 10 B. other reagents; and
- 11 C. directions for use of said kit.

29. A test kit for demonstrating the presence of a receptor recognition factor in a eukaryotic cellular sample, comprising:

- A. a predetermined amount of a receptor recognition factor, said receptor recognition factor having the following characteristics: apparent direct interaction with the ligand-bound receptor and activation of one or more transcription factors capable of binding with a specific gene; an activity demonstrably unaffected by the presence or concentration of second messengers; direct interaction with tyrosine kinase domains; and a perceived absence of interaction with G-proteins;
- B. a predetermined amount of a specific binding partner of said receptor recognition factor;
- C. other reagents; and
- D. directions for use of said kit;

wherein either said receptor recognition factor or said specific binding partner are detectably labelled.

1 30. The test kit of Claim 28 or 29 wherein said labeled immunochemically
2 reactive component is selected from the group consisting of polyclonal antibodies
3 to the receptor recognition factor, monoclonal antibodies to the receptor
4 recognition factor, fragments thereof, and mixtures thereof.

1 31. A method of preventing and/or treating cellular debilitations, derangements
2 and/or dysfunctions and/or other disease states in mammals, comprising
3 administering to a mammal a therapeutically effective amount of a material
4 selected from the group consisting of a receptor recognition factor, an agent
5 capable of promoting the production and/or activity of said receptor recognition
6 factor, an agent capable of mimicking the activity of said receptor recognition
7 factor, an agent capable of inhibiting the production of said receptor recognition
8 factor, and mixtures thereof, or a specific binding partner thereto, said receptor
9 recognition factor having the following characteristics:

- 10 a) apparent direct interaction with the ligand-bound receptor and
11 activation of one or more transcription factors capable of binding with a specific
12 gene;
13 b) an activity demonstrably unaffected by the presence or concentration
14 of second messengers;
15 c) direct interaction with tyrosine kinase domains; and
16 d) a perceived absence of interaction with G-proteins.

1 32. The method of Claim 31 wherein said disease states include chronic viral
2 hepatitis, hairy cell leukemia, and tumorous conditions.

1 33. The method of Claim 31 wherein said receptor recognition factor is
2 administered to modulate the course of therapy where interferon is being
3 administered as the primary therapeutic agent.

1 34. The method of Claim 31 wherein said receptor recognition factor is
2 administered to modulate the course of therapy where interferon is being co-
3 administered with one or more additional therapeutic agents.

1 35. A pharmaceutical composition for the treatment of cellular debilitation,
2 derangement and/or dysfunction in mammals, comprising:
3 A. a therapeutically effective amount of a material selected from
4 the group consisting of a receptor recognition factor, an agent capable of
5 promoting the production and/or activity of said receptor recognition factor, an
6 agent capable of mimicking the activity of said receptor recognition factor, an
7 agent capable of inhibiting the production of said receptor recognition factor, and
8 mixtures thereof, or a specific binding partner thereto, said receptor recognition
9 factor having the following characteristics: apparent direct interaction with the
10 ligand-bound receptor and activation of one or more transcription factors capable
11 of binding with a specific gene; an activity demonstrably unaffected by the
12 presence or concentration of second messengers; direct interaction with tyrosine
13 kinase domains; and a perceived absence of interaction with G-proteins; and
14 B. a pharmaceutically acceptable carrier.

1 36. A receptor recognition factor implicated in the transcriptional stimulation of
2 genes in target cells in response to the binding of a specific polypeptide ligand to
3 its cellular receptor on said target cell, said receptor recognition factor having the
4 following properties:
5 a) it is present in cytoplasm;
6 b) it undergoes tyrosine phosphorylation upon treatment of cells with
7 IFN α ;
8 c) it activates transcription of an interferon stimulated gene;
9 d) it stimulates either an ISRE-dependent or a gamma activated site
10 (GAS)-dependent transcription in vivo;
11 e) it interacts with IFN α cellular receptors, and

12 f) it undergoes nuclear translocation upon stimulation of the IFN cellular
13 receptors with IFN α .

1 37. A receptor recognition factor implicated in the transcriptional stimulation of
2 genes in target cells in response to the binding of an interferon or interferon-
3 related polypeptide ligand to its cellular receptor on said target cell, said receptor
4 recognition factor having the following properties:

5 a) it is present in vivo in mammalian cytoplasm before activation of
6 cellular IFN receptors;

7 b) it contains tyrosine sites that are phosphorylated in response to IFN
8 stimulation of IFN receptors;

9 c) it has a molecular weight selected from the group consisting of 48kD,
10 84kD, 91kD and 113kD, or an amino acid sequence selected from the group
11 consisting of SEQ ID NO:10 and SEQ ID NO:12, and

12 d) when phosphorylated, it recognizes an ISRE in the cell nucleus.

1 38. The receptor recognition factor of either of Claims 36 or 37 in
2 phosphorylated form.

1 39. An antibody which recognizes a phosphorylated ISGF3 polypeptide or a
2 fragment thereof in phosphorylated form.

1 40. An antibody produced by injecting a substantially immunocompetent host
2 with an antibody-producing effective amount of an ISGF3 polypeptide, and
3 harvesting said antibody, said ISGF3 polypeptide having the following properties:

4 a) it has a molecular weight of about 48kD, 84Kd, 91 Kd or 113kD or an
5 amino acid sequence selected from the group consisting of SEQ ID NO:10 and
6 SEQ ID NO:12;

7 b) it can be isolated from mammalian cytoplasm;

8 c) it contains tyrosine residues that are subject to phosphorylation in vivo
9 upon treatment of cells with IFN α ;

- 10 d) it can activate transcription of an interferon stimulated gene in vivo;
- 11 e) it can stimulate ISRE-dependent transcription in vivo;
- 12 f) it can interact with IFN α cellular receptors, and
- 13 g) it can undergo nuclear translocation upon stimulation of IFN cellular
- 14 receptors with IFN α .

1 41. The antibody of either of Claims 39 or 40 which is monoclonal.

1 42. The antibody of either of Claims 39 or 40 which is polyclonal.

1 43. A recombinant virus transformed with the DNA molecule, or a derivative
2 or fragment thereof, in accordance with Claim 14.

1 44. A recombinant virus transformed with the DNA molecule, or a derivative
2 or fragment thereof, in accordance with Claim 15.

1 45. A method of enhancing IFN α activity in a mammal in need of such
2 treatment, comprising administering to said mammal an effective amount of a
3 compound which (a) enhances the phosphorylation of intracellular ISGF3 proteins
4 to form ISGF3-protein phosphates, or (b) inhibits the activity of a phosphatase
5 enzyme which would otherwise reduce the level of phosphorylated ISGF3 proteins.

1 46. A method of treating (a) chronic viral hepatitis or (b) hairy cell leukemia,
2 in a mammal in need of such treatment, comprising administering to said mammal
3 an effective amount of a compound which (a) enhances the phosphorylation of
4 ISGF3 proteins, or (b) decreases the level of phosphate removal from
5 phosphorylated ISGF3 proteins.

1 47. The method of Claim 45 wherein the activity of exogenous IFN α is
2 enhanced.

- 1 48. The method of Claim 45 wherein the activity of endogenous IFN α is
2 enhanced.
- 1 49. The method of Claim 47 wherein the compound and IFN α are administered
2 concurrently to the mammal in need of such treatment.
- 1 50. A method of determining the interferon-related pharmacological activity of
2 a compound comprising:
3 administering the compound to a mammal;
4 determining the level of phosphorylated ISGF3 proteins present; and
5 comparing the level of ISGF3 protein-phosphate to a standard.
- 1 51. In a method of treating hepatitis or leukemia in a mammal, wherein IFN α
2 is administered in an amount effective for treating such hepatitis or leukemia, the
3 improvement comprising administering to said mammal an ISGF3 protein or a
4 derivative thereof in an amount effective for enhancing the activity of said IFN α .
- 1 52. The method of Claim 51 wherein a derivative of said ISGF3 protein is
2 administered.
- 1 53. The method of Claim 51 wherein an ISGF3 protein is administered, having
2 a molecular weight of about 48 kD, 84kD, 91kD or 113kD.
- 1 54. The method of Claim 52 wherein the derivative is a phosphorylated ISGF3
2 protein.
- 1 55. The recombinant DNA molecule of Claim 16 comprising plasmid pGEX-
2 3X, clone E3 or plasmid pGEX-3X, clone E4.
- 1 56. An antisense nucleic acid against a receptor recognition factor mRNA
2 comprising a nucleic acid sequence hybridizing to said mRNA.

- 1 57. The antisense nucleic acid of Claim 56 which is RNA.
- 1 58. The antisense nucleic acid of Claim 56 which is DNA.
- 1 59. The antisense nucleic acid of Claim 56 which binds to the initiation codon
2 of any of said mRNAs.
- 1 60. A recombinant DNA molecule having a DNA sequence which, on
2 transcription, produces an antisense ribonucleic acid against a receptor recognition
3 factor mRNA, said antisense ribonucleic acid comprising an nucleic acid sequence
4 capable of hybridizing to said mRNA.
- 1 61. A receptor recognition factor-producing cell line transfected with the
2 recombinant DNA molecule of Claim 60.
- 1 62. A method for creating a cell line which exhibits reduced expression of a
2 receptor recognition factor, comprising transfecting a recognition factor-producing
3 cell line with a recombinant DNA molecule of claim 60.
- 1 63. A ribozyme that cleaves receptor recognition factor mRNA.
- 1 64. The ribozyme of Claim 63 which is a Tetrahymena-type ribozyme.
- 1 65. The ribozyme of Claim 63 which is a Hammerhead-type ribozyme.
- 1 66. A recombinant DNA molecule having a DNA sequence which, upon
2 transcription, produces the ribozyme of claim 63.
- 1 67. A receptor recognition factor-producing cell line transfected with the
2 recombinant DNA molecule of claim 66.

- 1 68. A method for creating a cell line which exhibits reduced expression of a
- 2 receptor recognition factor, comprising transfecting a recognition factor-producing
- 3 cell line with the recombinant DNA molecule of claim 63.